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16. ABSTRACT

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THE POSTNATAL EFFECTS OF PRENATAL EXPOSURE TO LOW DOSES OF NITROFEN (2,4-DICHLOROPHENYL-*p*-NITROPHENYL ETHER) IN SPRAGUE—DAWLEY RATS

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SUMMARY

Nitrofen was administered to pregnant Sprague—Dawley rats by gavage on days 8—16 of gestation at 5 different dose levels — 0, 0.46, 1.39, 4.17 and 12.5 mg/kg/day. Diaphragmatic hernias were found in pups that died immediately after birth at the 3 highest dose levels. At the 1.39-mg/kg dose level 3 of the 4 pups examined had diaphragmatic hernias, at the 4.17-mg/kg dose level 2 out of 3 pups had diaphragmatic hernias, and at the 12.5-mg/kg dose level all 5 pups found dead had diaphragmatic hernias. Locomotor activity of the offspring was measured on postnatal days 17 and 24, and hyperactivity was evident at the 3 highest dose levels. However, when the rats were later tested at 45, 49 and 90 days of age they had apparently recovered from this earlier hyperactivity. In the female rat, nitrofen did not delay the onset of puberty as measured by the age of vaginal opening or the age at first estrus. At necropsy of the offspring which began on postnatal day 133, Harderian gland weight reduction and hydronephrosis were seen at the 4.17- and 12.5-mg/kg dose levels, while no effects were found in body, liver, testes, seminal vesicle, kidney, or lung weights.

Results of the present study and earlier studies demonstrate that rats are more sensitive than mice to the teratogenic effect of nitrofen (Gray et al., Science, 215 (1982) 293 and Gray et al., Toxicol. Appl. Pharmacol., 67 (1983) 1). In general, nitrofen affects the same organ systems in rats as

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it does in mice, but the rank order of sensitivity of these effects differs from those described earlier in the mouse by Gray et al. (Toxicol. Appl. Pharmacol., 67 (1983) 1).

Key words: Nitrofen; Diaphragm; Hyperactivity; Thyroxine; Hydronephrosis

INTRODUCTION

Nitrofen is a diphenyl ether compound (2,4-dichlorophenyl-p-nitrophenyl ether) which has been used as a pre- or post-emergent herbicide to control weeds and grasses in agriculture fields and along right of ways [3]. Although nitrofen's exact mechanism of herbicidal action is unknown, it is thought that nitrofen produces its phytotoxic effects by competing with ADP to inhibit energy transfer in the ATP-synthetase reaction [4]. In the environment nitrofen may be decomposed by sunlight, or as one study showed in an aqueous suspension approximately 90% of the nitrofen is photo-decomposed to 2,4-dichlorophenol and 4-nitrophenol after 4 weeks of exposure to sunlight [5]. Exposure to nitrofen may occur in herbicide production workers or farmers through dermal contact or inhalation, while the general population may be exposed orally through residues that may remain on food crops [6].

Early toxicological studies on the effects of nitrofen showed that its toxicity to adult rats was relatively low having an acute oral LD_{50} of approximately 2600 mg/kg [7,8]. In contrast to this low toxicity to adult rats, the calculated LD_{50} for neonatal Long-Evans rats exposed in utero on day 11 of gestation was found to be approximately 116 mg/kg. In these newborn rat pups, cardiac malformations and diaphragmatic hernias were determined to be the leading causes of death [9]. Another serious, but not necessarily lethal abnormality observed in the neonates was hydronephrosis; for example, in one study 50% of the surviving pups exhibited hydronephrosis when they were examined on postnatal day 35 as a result of exposure on day 11 of gestation to 200 mg/kg nitrofen which is approximately 1.7 times the neonatal LD_{50} dose level [9].

The offspring of mice dosed by gavage on days 8—12 of gestation with 200 mg/kg/day had excessive postnatal mortality, and the survivors exhibited abnormalities including anophthalmia, hydrocephaly, and malocclusions. Lower doses from 150 to 50 mg/kg/day given to pregnant mice for longer durations (days 7—17 of gestation) also increased neonatal mortality through postnatal day 3, while doses ranging from 50 to 6.25 mg/kg/day given on days 7—17 of gestation caused organ weight reduction (liver, lungs, seminal vesicles, and Harderian glands) and other abnormalities such as delayed puberty in the females and a decreased fertility rate. Also , approximately 65% of the mice exposed to 50 mg/kg of nitrofen on days 7—17 of gestation were missing one or both Harderian glands [1,2].

In mice the lowest dose level which caused reduced pup survival to day 3 was 50 mg/kg/day given on days 7—17 of gestation [2] whereas in the

rat 12.5 mg/kg/day given on days 8–16 of gestation caused a 30% reduction in the number of pups surviving to day 2 [10]. Therefore, this study was designed with 3 objectives in mind: (1) to examine the effects of prenatal nitrofen exposure in rats at dose levels where excessive neonatal mortality does not mask the nonlethal postnatal defects; (2) to determine the lowest dose level for each particular defect; and (3) to compare the effects and the doses at which these effects occur in the rat to those already described in the mouse.

MATERIALS AND METHODS

Animals

Sixty, 90-day-old, time pregnant, Sprague–Dawley CD rats were received from Charles River Breeding Laboratory, Wilmington, MA, on day 3 of gestation, day 1 of gestation being the day a sperm positive vaginal smear was seen. Upon receipt the rats were housed individually in clear plastic cages (20 × 25 × 47 cm) with heat-treated laboratory grade pine shavings (Northeastern Products Corp., Warrensburg, NY) as litter, and Purina rodent laboratory chow (5001) and water were given ad libitum. The rats were kept on a 12 h light/12 h dark cycle with the light cycle beginning at 0600 h (EST), and the temperature in the animal room was maintained at 20–24°C with a relative humidity of 40–50%.

Dosing regimen

Nitrofen (recrystallized from technical grade Nitrofen with a m.p. of 70.5–71.5°C and a purity of 99.59%) was dissolved in corn oil and administered via oral gavage at the rate of 0.2 ml/day on days 8–16 of gestation. The dosing solutions were prepared on the basis of the average weight of all rats on day 7 of gestation. The dosing regimen was as follows: 13 dams received 0.0 mg nitrofen/kg body wt/day in 0.2 ml corn oil; 13 – 0.46 mg/kg/day; 13 – 1.39 mg/kg/day; 13 – 4.17 mg/kg/day; and 8 – 12.5 mg/kg/day. The highest dose (12.5 mg/kg) was chosen because it has been reported that 69 ± 7% of the pups survived to day 2 when the dams were given 12.5 mg nitrofen/kg body wt on day 8–16 of gestation, while lower dose levels did not produce a significant reduction in survivorship to day 2 [10]. The remaining doses (4.17, 1.39, and 0.46 mg/kg) were found by multiplying the highest dose by one-third, one-ninth and one twenty-seventh, respectively. Before and after dosing (days 7 and 17 of gestation) the dams were weighed to determine if there was a treatment effect on maternal weight gain.

Pup viability and eye opening

After parturition any dead pups were necropsied to determine the cause of death. On postnatal days 0, 1, 2, and 6 (postnatal day 0 being the day of birth) the number of pups/litter and the litter weight were recorded to determine if there was a treatment effect on litter size, average pup weight, or pup

viability. On day 24 of gestation any dams that had not delivered were necropsied to determine if they were not pregnant or if resorption had occurred. The percent of eyes open for each dosage group was calculated on postpartum day 16, since it has been reported that mice may exhibit a significant delay in eye opening following in utero nitrofen exposure [2].

Behavioral tests

It has also been reported that mice exhibit dose related hyperactivity during certain developmental periods following in utero nitrofen exposure [11]. To test this hypothesis a total of 86 pups (20 controls, 16 at the 0.46-mg/kg dose level, 16 at the 1.39-mg/kg dose level, 18 at the 4.17-mg/kg dose level, and 16 at the 12.5-mg/kg dose level) were run in figure 8 activity mazes on days 17 and 24 with equal numbers of males and females at each dose level [12]. The number of photocell beam interruptions (8 photocells/maze) was recorded for each animal for a period of 60 min. On days 45 and 49, 64 naive pups (22 controls, 8 — 0.46 mg/kg, 10 — 1.39 mg/kg, 12 — 4.17 mg/kg and 12 — 12.5 mg/kg) were run in the activity mazes with equal numbers of males and females at each dose level, and again on day 90, 32 naive males (8 controls, 6 — 0.46 mg/kg, 7 — 1.39 mg/kg, 5 — 4.17 mg/kg, and 6 — 12.5 mg/kg) were run in the activity mazes for 1 h each. Also, in order to verify the hyperactivity seen in this experiment a second Block was conducted using only the 0- and 12.5-mg/kg/day dosage groups, and locomotor activity was observed in 24 controls and 16—12.5-mg/kg pups on days 17 and 24 as in Block 1.

Reproductive tests

The pups were weaned on day 29, and housed in unisexual littermate groups of 2 or 3 pups/cage. The females were checked daily from days 32 to 44 to determine age at vaginal opening, and the age at first estrus as indices of the onset of puberty. The onset of sexual maturity has been shown to be highly correlated with vaginal patency and initial ovulation in the female laboratory rat [13].

On day 54 the rats were put into non-littermate breeding pairs according to treatment, and housed 1 pair/cage. As soon as a female gave birth, her pups were counted and then disposed of. This data collection continued until all the females had sufficient time to deliver 3 litters.

Necropsy

After the breeding data were collected and beginning at 133 days of age, the males were asphyxiated with CO_2, and the body, seminal vesicles, testes, liver, right kidney, lungs and Harderian glands were weighed. The kidneys were sliced to check for hydronephrosis, the kidney cortex diameter was measured, and the eyes were checked for the presence of porphyrin rings.

Data analysis

The data were analyzed using analysis of variance available in the Statis-

tical Analysis System [14]. Significant effects were subsequently tested using *t*-tests, and reanalyzed using linear regression to determine if these effects were dose related. The unit of analysis for all preweanling data was the litter mean, since in previous studies we have not observed any litter effects on these parameters, while the individual animal was the unit of analysis for all post-weanling data.

RESULTS

Prenatal development

Maternal viability and weight change from days 7 to 17 of gestation was not affected by nitrofen administration at any dose level. Two females died before parturition; however, one of these rats was sick prior to dosing, and the other rat found dead on day 22 of gestation probably died as a result of delivery complications. The day 3 postpartum necropsy of any females that had not delivered showed that 3 control rats, 2 rats at the 0.46-dose level, 1 at 1.39-dose level, 1 at the 4.17-dose level, and 1 at the 12.5-dose level were not pregnant. At the 1.39-dose level 1 female had normal fetuses, but had not delivered by the time of the necropsy. At the 4.17-dose level 1 female was found to have resorbed her pups, and another dam gave birth only to dead pups. All maternal data can be found in Table I.

TABLE I

EFFECTS OF NITROFEN ADMINISTRATION ON PREGNANT RATS DOSED ON DAYS 8—16 OF GESTATION

Nitrofen dose (mg/kg/day)	0.0	0.46	1.39	4.17	12.5
No. dams on treatment	13	13	13	13	8
No. dams not pregnant[a]	3	2	1	1	1
No. dams with dystocia[b]			1		
No. of dams which resorbed pups				1	
No. dams that died before parturition[c]		2			
No. dams that delivered only dead pups				1	
Mean dam weight change in grams from days 7—17 ± S.D.	81.22 ±10.96	72.44 ±13.25	76.64 ±10.83	80.60 ±16.48	79.00 ± 9.31
No. litters for which data analyzed	10	9	11	10	7

[a] At necropsy no implantation sites were found.

[b] At necropsy no resorptions were found and the fetuses appeared normal, however, this female was unable to deliver her pups.

[c] One of these females was sick prior to dosing and was found dead on day 16 of gestation; the other female appeared healthy and had a normal weight gain during pregnancy but was found dead on day 22 of gestation.

Postnatal development

In earlier studies in which Sprague—Dawley rats were dosed by gavage on approximately the same days of gestation, the number of control pups/litter was found to average 10.8 pups/litter for 18 litters [10] and 10.3 pups/litter for 21 litters [15] as compared to 12.1 pups/litter for 9 litters in this experiment. Also one study [15] found the mean control pup weight on day 1 to be approximately 6.55 g as compared to a mean control pup weight of 6.9 g in this experiment.

A necropsy of any pups that died shortly after birth demonstrated that prenatal nitrofen exposure caused diaphragmatic hernias at the 1.39-, 4.17-, and 12.5-mg/kg dose levels (Table II). No diaphragmatic hernias were found in any dead pups at the 0.0-mg/kg or 0.46-mg/kg dose levels.

Upon analysis of the day 0—6 postpartum pup data, it was found that the mean number of total pups/litter on day 0 (day of birth) at the 0.46- and the 4.17-dose levels, and the mean number of live pups on days 1, 2 and 6 at

TABLE II

EFFECTS OF NITROFEN ADMINISTERED ON DAYS 8—16 OF GESTATION ON 0—6-DAY-OLD PUPS (DAY 0 = DAY OF BIRTH)[a]

	Dose level (mg/kg/day)				
	0.0	0.46	1.39	4.17	12.5
	No. of litters				
	9	9	11	10	7
No. of pups/litter day 0	12.1	8.2[b]	10.5	9.2[c]	10.1
No. pups live on day 1	11.9	8.0[b]	9.9	8.9[c]	8.4[c]
No. pups live on day 2	11.8	8.0[b]	9.8	8.9[c]	8.4[c]
No. pups live on day 6	11.6	7.7[b]	9.3	8.5[c]	8.1[c]
$\bar{x}$ APW day 1[d]	6.9	7.2	7.0	6.8	6.7
$\bar{x}$ APW day 2[d]	8.1	8.2	7.8	8.0	7.7
% Survival to day 2	98%	98%	93%	97%	83%
% Survival to day 6	96%	89%	88%	84%	78%[c]
No. dead pups recovered day 0	4	2	4	3	5
No. litters from which dead pups recovered (day 0)	2	2	3	3	3
No. pups with diaphragmatic hernias	0	0	3	2	5
No. litters with pups having diaphragmatic hernias	0	0	3	2	3

[a]Data were analyzed by analysis of variance and a *t*-test and presented as the mean.
[b]Significantly different from control value, $P < 0.01$.
[c]Significantly different from control value, $P < 0.05$.
[d]The average pup weight (APW) for each litter was obtained by dividing the litter weight by the number of pups in that litter. The mean of these values was then calculated for all the litters in each treatment group.

the 0.46-, 4.17- and the 12.5-dose levels were significantly reduced, but linear regression indicated that these effects were not dose related. However, the percent of pups surviving to day 6 at the 12.5-mg/kg dose level was significantly reduced when analyzed by linear regression. On day 16 there was no delay in eye opening as all pups had both eyes open at this time.

Behavioral data

The day 17 and 24 locomotor activity data of Blocks 1 and 2, was pooled and the Block, age, sex, dose effects and their interactions were determined. Only prenatal exposure to nitrofen significantly affected locomotor activity; the variables age, sex, and block were not significant (Table III). The 1.39- and 4.17-dose levels ($P < 0.05$) and the 12.5-dose level ($P < 0.01$) were hyperactive as compared to the controls at this age. Analysis of the 45-, 49-, and 90-day diurnal maze activity data showed that the rats had apparently recovered from their hyperactivity as there was no significant difference in the activity levels of the control and treated animals (Table III).

The locomotor activity level for control Sprague—Dawley rats observed in an earlier experiment [15] for 45-min intervals on days 16 and 20 was found to average 282, while the activity level for the control rats in this

TABLE III

THE EFFECT OF PRENATAL EXPOSURE TO NITROFEN (DAYS 8—16 OF GESTATION) ON THE DEVELOPMENT OF LOCOMOTOR ACTIVITY IN SPRAGUE—DAWLEY RATS[a]

Nitrofen dose (mg/kg/day)		0.0	0.46	1.39	4.17	12.5
Block 1	day 17	251 ± 31 (12)	305 ± 38 (8)	323 ± 38 (8)	273 ± 34 (10)	345 ± 34[b] (10)
	day 24	266 ± 38 (8)	307 ± 38 (8)	363 ± 38 (8)	421 ± 38[c] (8)	359 ± 44 (6)
Block 2	day 17	234 ± 22 (13)	—	—	—	306 ± 23[b] (11)
	day 24	294 ± 25 (11)	—	—	—	377 ± 32[b] (5)
Block 1 & 2	days 17 & 24	264 ± 16 (44)	304 ± 29 (16)	341 ± 29[b] (16)	345 ± 28[b] (18)	341 ± 20[c] (32)
Block 2	days 45 & 49	682 ± 66 (22)	632 ± 63 (8)	638 ± 61 (10)	660 ± 69 (12)	689 ± 59 (12)
Block 2	day 90	749 ± 53 (8)	734 ± 50 (6)	648 ± 63 (7)	719 ± 95 (5)	748 ± 65 (6)

[a] Values represent the number of photocell beam interruptions recorded in a 1-h period in a figure 8 maze (8 photocells/maze). Values are presented as the mean ± the standard deviation of the mean, and the numbers in parentheses equal the number of animals at that dose level.

[b] Significantly different from control value, $P < 0.05$.

[c] Significantly different from control value, $P < 0.01$.

experiment measured over 60-min intervals on days 17 and 24 was 264. Also, the 90-day control male locomotor activity seen in this earlier experiment was 750 as compared to an activity level of 749 for the 90-day-old control males in this experiment.

Reproductive data

The onset of puberty in the treated female rats was not affected as there was no significant difference in the day of vaginal opening or first estrus between the treated females and the controls. Likewise, the breeding data showed that there was no significant difference in litter size over 3 breeding cycles between the controls and treated animals.

Necropsy data

The necropsy performed on the rats on days 133—161 showed that in the males, Harderian gland weight was affected at both the 4.17-mg/kg and 12.5-mg/kg dose levels (Table IV). At the 12.5-mg/kg dose level 13 animals were observed to have porphyrin rings around 1 or both eyes, and hydronephrosis was seen in 6 animals at this level. At the 4.17-mg/kg dose level 3 animals also exhibited hydronephrosis. The kidneys were determined to be hydronephrotic when the pelvic area was hollow, greatly enlarged and the extension of the papilla into the pelvic area was minimal or almost non-existant as a result of fluid pressure. It should be noted that although enlargement of the renal pelvis is common in older rats, in this study hydronephrosis was not observed in any of the control animals or the 2 lower dose levels, and the kidneys described as being hydronephrotic at the 2 highest dose levels represent very severe cases of this abnormality. Other parameters measured at necropsy which were not affected by treatment were: body weight, testes weight, seminal vesicle weight, liver weight, lung weight, right kidney weight, and kidney cortex diameter.

DISCUSSION

In an earlier cross-fostering experiment it was demonstrated that mice exposed to nitrofen only during lactation were unaffected, therefore it is likely that the postnatal effects seen in this experiment are due only to prenatal exposure [2].

In the present experiment, when nitrofen was administered orally to pregnant rats on days 8—16 of gestation, it caused a linear decrease in survival to postnatal day 6, with only the 12.5-mg/kg dosage group being significantly less than the control value. At the 3 highest dose levels, 75% (1.39), 66% (4.17), and 100% (12.5) of the pups found dead on day 0 had diaphragmatic hernias, which strongly suggests this malformation is one of the primary causes of early neonatal mortality.

As mentioned in the results the mean litter size at birth at the 0.46- and the 4.17-mg/kg dose levels, and the mean number of pups/litter on days 1, 2, and 6 at the 0.46-, 4.17-, and 12.5-mg/kg dose levels were significantly

TABLE IV

THE EFFECTS OF PRENATAL NITROFEN SEEN AT NECROPSY OF 130—160-DAY-OLD MALE RATS

Dose (mg/kg/day)	0.0	0.46	1.39	4.17	12.5
Body weight[a]	640.9 ± 76 (24)	652.9 ± 70 (25)	621.2 ± 53 (25)	644.9 ± 66 (22)	655.3 ± 59 (17)
Testes weight[a]	3.85 ± 0.29 (6)	3.39 ± 0.61 (6)	3.71 ± 0.22 (6)	3.86 ± 0.42 (6)	3.90 ± 0.26 (6)
Seminal vesicle weight[a]	1.96 ± 0.29 (25)	1.98 ± 0.33 (25)	1.97 ± 0.38 (25)	1.90 ± 0.31 (22)	1.90 ± 0.35 (17)
Liver weight[a]	27.01 ± 4.46 (6)	27.78 ± 3.96 (6)	24.83 ± 2.76 (6)	30.79 ± 5.12 (6)	25.72 ± 3.90 (6)
Right kidney weight[a]	2.40 ± 0.17 (6)	2.47 ± 0.22 (6)	2.31 ± 0.28 (6)	2.70 ± 0.21 (6)	2.39 ± 0.24 (6)
Lung weight[a]	1.80 ± 0.19 (6)	1.85 ± 0.11 (6)	1.98 ± 0.42 (6)	2.13 ± 0.23 (6)	1.88 ± 0.13 (6)
Harderian gland weight[a]	0.392 ± 0.06 (25)	0.385 ± 0.05 (25)	0.391 ± 0.04 (25)	0.358 ± 0.07[c] (22)	0.303 ± 0.04[d] (17)
Hydronephrotic kidneys[b]	0	0	0	3	6
Porphyrin rings around 1 or both eyes[b]	0	0	0	0	13

[a] Variables represent the mean weight in grams ± the standard deviation; the number in parentheses equals the number of observations.
[b] Variables represent the number of animals with hdyronephrotic kidneys or porphyrin rings.
[c] Significantly different from control mean, $P < 0.05$
[d] Significantly different from control mean, $P < 0.01$.

reduced as compared to the controls. Based on earlier studies using rats and mice we expected that nitrofen would not cause a reduction in the number of pups born, but that postnatal mortality would be increased by treatment. For example, in one study when mice were dosed on days 7—17 of gestation with 100 mg/kg nitrofen there was no significant reduction in the number of pups born, but by postnatal day 3 more than 50% of the offspring had died [2]. For this reason and because in this experiment the reduction in the number of pups on day 0 at the 0.46- and 4.17-mg/kg dose level was not dose related when analyzed by linear regression, we feel that this phenomena is not significant even though at present we are at a loss to explain why these values were reduced.

Additional developmental landmarks such as eye opening, vaginal opening, and age at first estrus were not affected by nitrofen at the doses given in this experiment, although they were affected in mice in studies in our lab using higher doses of nitrofen. On days 17 and 24 the 1.39-, 4.17-, and 12.5-mg/kg dose level animals exhibited statistically significant dose related hyperactivity. However, this phenomena disappeared when the animals were again tested at 45, 49, and 90 days of age. Transient hyperactivity has also been observed in the offspring of mice treated with 200 mg/kg/day nitrofen on days 8—12 of gestation or with 100 and 200 mg/kg/day administered on days 8 and 9 of gestation [11].

At necropsy Harderian gland weight was significantly reduced at the 4.17- and 12.5-mg/kg dose level, where $P < 0.05$ at the 4.17-mg/kg dose level, and $P < 0.01$ at the 12.5-mg/kg dose level, and chromodacryorrhea which involved 1 or both eyes was observed in 13 animals at the 12.5-mg/kg dose level. This data agrees with a previous experiment in which rats were dosed at 12.5 mg/kg on days 8—16 of gestation and there was no delay in the age at eye opening but the Harderian glands were affected [1].

In the rat the Harderian glands are bilobed exocrine glands located in the orbit behind the eyeballs. A secretion from these glands empties onto the inner surface of the nictating membrane and serves primarily to protect and lubricate the cornea. It has been pointed out that thyroidectomy causes Harderian gland regression, while the administration of thyroxine to neonatal animals accelerates the development of this gland [1]. It has also been observed that in the rat an increase in thyroxine secretion and the growth of the Harderian gland are developmentally related, and that nitrofen and the thyroid hormones are all diphenyl ethers [1]. A later study showed that prenatal exposure to nitrofen does in fact lower serum thyroxine levels in adult female mice, and the authors suggested that some of the postnatal alterations resulting from prenatal nitrofen exposure might be related to altered thyroid physiology [16].

Also at necropsy 3 animals at the 4.17-mg/kg dose level and 6 animals at the 12.5-mg/kg dose level had hydronephrotic kidneys. In another study in which rats were exposed to 4.17, 12.5, and 25.0 mg/kg nitrofen on days 8—16 of gestation renal function was affected at all dose levels, and although animals with hydronephrotic kidney faired poorly on these renal function

TABLE V

COMPARISON BETWEEN SPRAGUE—DAWLEY RATS IN THE PRESENT STUDY AND CD-1 MICE [1,2] OF THE POSTNATAL DEFECTS PRODUCED BY PRENATAL NITROFEN ADMINISTRATION

Nitrofen dose (mg/kg/day)	Rats (dosed days 8—16 of gestation)[a]					Mice (dosed days 7—17 of gestation)[a]							
	0.0	0.46	1.39	4.17	12.5	0	6.25	12.5	25	50	100	150	200
Number of pups live day 1	11.9	8.0[b]	9.9	8.9[c]	8.4[c]	10.7	10.0	11.6	10.7	10.6	9.6	8.7[c]	7.6[b]
% of dead pups recovered with diaphragmatic hernias	0	0	75	66	100	0	—	0	0	0	0	0	6
Paired Harderian gland wt in mg for males	392	385	391	358[c]	303[b]	41.9	38.7[b]	36.8[b]	31.9[b]	28.8[b]	—	—	—
Locomotor activity days 17 + 24 in rats and days 22—24 in mice[d]	264	304	341[b]	345[b]	341[b]	648	—	—	—	579	597	—	—[e]

[a] Values are means with the exception of variable 2 (% of dead pups recovered)
[b] Significantly different from control mean, $P < 0.01$
[c] Significantly different from control mean, $P < 0.05$
[d] Values represent photocell beam interruptions in figure 8 mazes
[e] In another study mice exposed prenatally to 200 mg/kg/day nitrofen on days 8—12 of gestation exhibited hyperactivity when tested on days 22—24

tests, animals with normal appearing kidneys also showed evidence of renal dysfunction [10].

In Table V, which compares the effects of prenatal nitrofen administration between Sprague—Dawley rats and CD-1 mice, it can be seen that reduced pup viability on day 1, the number of pups with diaphragmatic hernias, and day 17—24 hyperactivity occur at lower doses in rats than in mice. However, Harderian gland weight reduction occurs at about the same dose levels in both species.

In summary, the significant postnatal defects seen in this experiment and the dose levels at which these effects were seen were as follows: diaphragmatic hernias — 1.39, 4.17, and 12.5 mg/kg; hyperactivity on days 17 and 24 — 1.39, 4.17, and 12.5 mg/kg; Harderian gland weight reduction — 4.17 and 12.5 mg/kg; hydronephrosis — 4.17 and 12.5 mg/kg; and reduced survivorship to postnatal day 6 — 12.5 mg/kg.

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